

International Journal Research Publication Analysis

Page: 01-19

PHYTOCHEMICAL SCREENING AND IN VITRO EVALUATION OF ANTI-UROLITHIATIC AND ANTI-INFLAMMATORY ACTIVITIES OF *CROTON SPARSIFLORUS* EXTRACT

K. Yuvarani^{*1}, P. Ananthika², R. Archand², V. Elakkiya², R. Pandiyan², K. Vimal²

¹ Associate Professor Department of Pharmacology,

² B. Pharm Final Year Students

Shree Krishna College of Pharmacy

Pakkiripalayam Village, Chengam, Tiruvannamalai-606709.

Article Received: 29 August 2026

Article Revised: 17 September 2026

Published on: 07 October 2026

*Corresponding Author: K. Yuvarani

Associate Professor Department of Pharmacology, Shree Krishna College of Pharmacy PakkiriPalayam Village, Chengam, Tiruvannamalai-606709.

DOI: <https://doi.org/10.1555/ijrpa.4225>

ABSTRACT

The present study was undertaken to investigate the phytochemical profile and in vitro antiurolithiatic and anti-inflammatory activities of the ethanolic extract of *Croton sparsiflorus* (EECS). The dried and powdered plant material was extracted with ethanol using the Soxhlet extraction method, yielding 22.5% w/w of extract. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides, and carbohydrates, whereas proteins and anthraquinones were absent. The antiurolithiatic potential of EECS was evaluated using calcium oxalate nucleation, crystal-growth, and aggregation assays at concentrations ranging from 100 to 1,000 µg/mL. The extract exhibited a concentration-dependent inhibitory effect in all three assays. At 1,000 µg/mL, EECS produced $78.91 \pm 2.04\%$ inhibition of nucleation, $75.62 \pm 1.96\%$ inhibition of crystal growth, and $73.84 \pm 1.88\%$ inhibition of crystal aggregation, compared with $91.48 \pm 1.27\%$, $89.76 \pm 1.34\%$, and $88.92 \pm 1.29\%$, respectively, for the standard drug. One-way ANOVA demonstrated highly significant differences among the tested concentrations for nucleation ($F = 1463.89$; $p = 1.15 \times 10^{-14}$), crystal growth ($F = 1407.31$; $p = 1.43 \times 10^{-14}$), and aggregation ($F = 1374.23$; $p = 1.60 \times 10^{-14}$). Anti-inflammatory activity was assessed using the egg albumin protein-denaturation assay. EECS showed an optical density of 0.129 and 71.26% inhibition of protein denaturation, whereas the standard drug produced an optical density of 0.146 and 67.48% inhibition. These findings

indicate that the ethanolic extract of *Croton sparsiflorus* possesses promising in vitro antiurolithiatic and anti-inflammatory activities, which may be attributed to the synergistic effects of its phytochemical constituents. Further isolation of active compounds, toxicity assessment, mechanistic studies, and in vivo investigations are warranted to establish its therapeutic potential.

KEYWORDS: *Croton sparsiflorus*, ethanolic extract, phytochemical screening, antiurolithiatic activity, calcium oxalate, nucleation inhibition, anti-inflammatory activity, protein denaturation.

1. INTRODUCTION

Urolithiasis is a common disorder of the urinary system characterized by the formation of solid crystalline masses, commonly known as urinary stones or calculi, within the kidneys or other parts of the urinary tract. The condition may involve the kidneys, ureters, urinary bladder and urethra. Urinary stone disease is considered a multifactorial disorder resulting from complex interactions between genetic, metabolic, dietary, environmental and lifestyle-related factors. Although small stones may pass spontaneously without causing significant symptoms, larger stones can produce severe renal colic, haematuria, urinary obstruction, infection and, in prolonged or complicated cases, impairment of renal function.

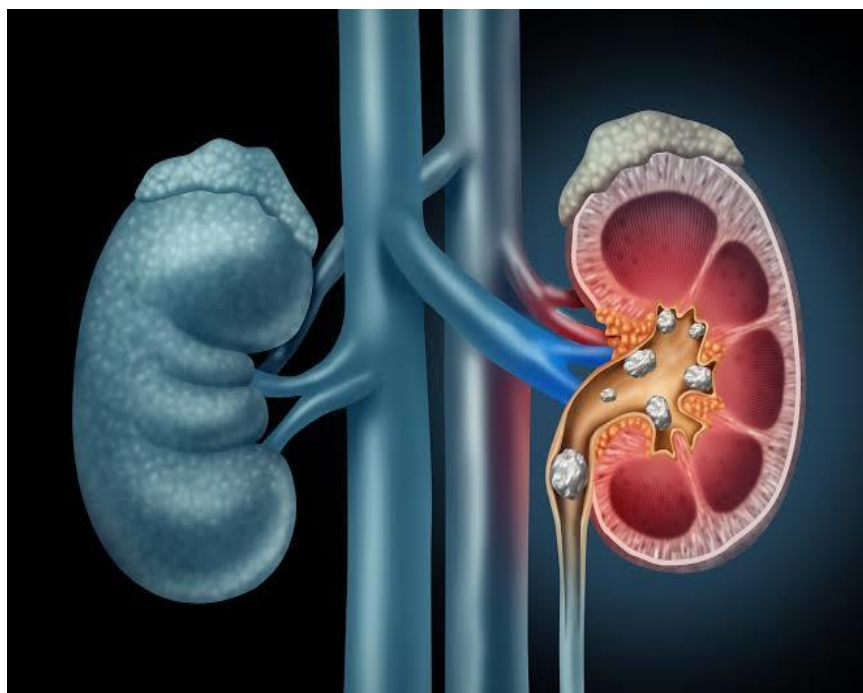


Fig: 1 Urolithiasis.

Inflammation is a complex and highly regulated protective response of living tissues to harmful stimuli such as infection, tissue injury, chemical irritation and cellular stress. It forms an important part of the body's innate defence system and helps eliminate harmful agents, remove damaged cells and initiate tissue repair. Although inflammation is normally beneficial and self-limiting, excessive or persistent inflammation can cause tissue damage and contribute to the development and progression of several pathological conditions.

The inflammatory response involves coordinated interactions between immune cells, blood vessels and chemical mediators. Following tissue injury or infection, inflammatory cells become activated and release mediators that regulate vascular permeability, leukocyte recruitment, pain, tissue injury and subsequent repair. Important mediators include vasoactive amines, eicosanoids, cytokines, chemokines and other signaling molecules.

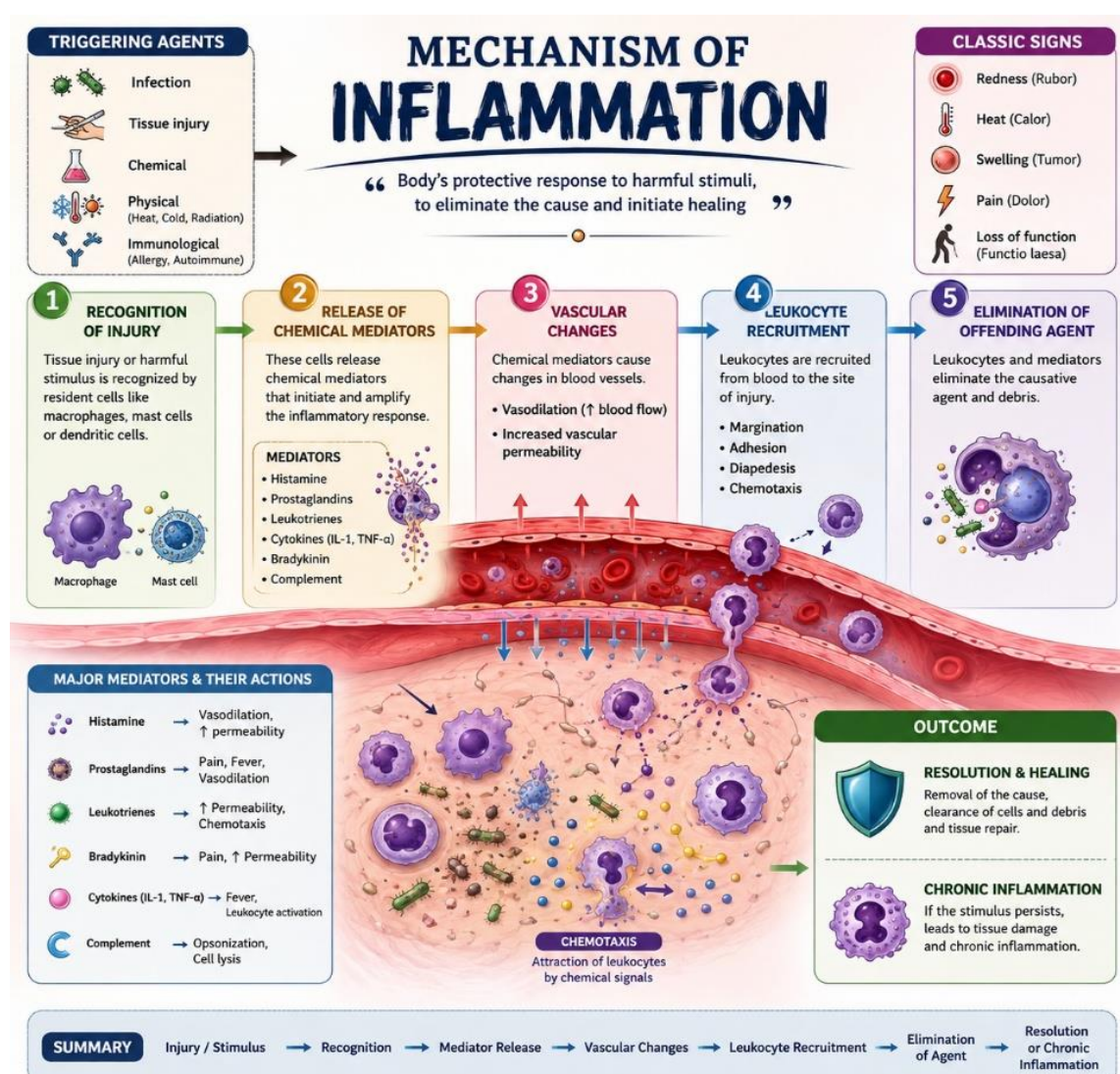


Fig: 2 Mechanism of Inflammation.

2. PLANT PROFILE

2.1 Botanical Description

Croton sparsiflorus Morong belongs to the family Euphorbiaceae and is described in the literature as a small annual herb. It commonly grows in disturbed habitats such as roadsides and open areas. The plant may reach approximately 1–2 feet in height. The leaves are arranged alternately and are approximately 3–5 cm long. They are lance-shaped and possess toothed margins.

The flowers are small and white and are arranged in terminal racemes approximately 3–8 cm long. The flowers have five sepals and five petals with numerous prominent stamens. The fruit is described as a small, oblong capsule with a warty surface. The plant has also been reported under the vernacular name “Ban Tulasi” in some Indian literature.

2.2 Taxonomy

The conventional taxonomic classification of the plant is:

Table: 1 Taxonomy.

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Angiosperms
Class	Eudicots
Order	Malpighiales
Family	Euphorbiaceae
Genus	<i>Croton</i>
Species	<i>Croton Sparsiflours</i> Morong

2.3 Morphological Features

The morphological characteristics of *C. sparsiflorus* can be described under the major plant parts.

Root: The root system provides anchorage to the plant and is responsible for the absorption of water and minerals from the soil.

Stem: The stem forms the main aerial axis of the plant and supports the leaves and reproductive structures. The surface and branching characteristics can be used as supporting features during identification.

Leaves: The leaves are simple and occur along the stem. Important diagnostic characters include their arrangement, shape, margin, apex, base and venation. The surface characteristics of the leaves may also be useful for identification.

Flowers: The flowers are small and occur in inflorescences. Floral characteristics, together with vegetative features, are useful for taxonomic identification.

Fruit and Seeds: The plant produces fruits containing seeds. The characteristics of the fruit and seed may provide additional features for botanical identification.



Fig: 3 Croton Sparsiflorus.

2.4 Distribution

The genus *Croton* has a wide distribution in tropical and subtropical regions. Different species are found across parts of Asia, Africa and the Americas. The genus shows considerable ecological diversity, with individual species occurring in different habitats.

The distribution of *C. sparsiflorus* should be described according to the authenticated species and the geographical region from which the plant material was collected. Environmental factors such as soil, temperature, rainfall and geographical location may influence the growth and phytochemical composition of medicinal plants.

2.5 Phytochemical & Its Importance

The genus *Croton* is recognized for its rich diversity of secondary metabolites. Various investigations have reported the occurrence of Alkaloids, Flavonoids, Phenolic compounds, Tannins, Terpenoids, Diterpenoids, Triterpenoids, Steroids and Glycosides in different species.

Table: 2 Major Phytochemical Constituents & Their Biological Significance.

PHYTOCHEMICAL	ROLE
Flavonoids (0.224%)	Antioxidant and anti-inflammatory potential
Phenolics (0.62%)	Antioxidant activity; may help reduce oxidative stress
Alkaloids (7.61%)	Various pharmacological activities, including reported anti-inflammatory effects
Saponins (15.2%)	May contribute to anti-inflammatory and other biological activities
Tannins (1.10%)	Antioxidant and tissue-protective potential
Terpenoids (NA)	Reported anti-inflammatory and other pharmacological activities
Steroids (NA)	Some plant steroids possess anti-inflammatory properties

3. MATERIALS AND METHODS

3.1 Collection of Plant Material

- The selected plant part of *Croton sparsiflorus* Morong was collected from a suitable location.
- The collected plant material was carefully examined for its morphological characteristics.
- The material was then subjected to shade drying at room temperature until a constant weight was obtained.
- A voucher specimen should be preserved and assigned an appropriate voucher number for future reference.
- previous studies on *C. sparsiflorus* have also used authenticated plant material for phytochemical and pharmacological investigations.

**Fig: 4 Collection of Plant Material.**



Fig: 5 Preparation of Plant Material.

3.2 Preparation of Plant Material

The dried plant material was separated from unwanted materials and mechanically powdered using a suitable grinder. The powdered material was passed through a suitable sieve to obtain a relatively uniform particle size. The powdered material was stored in a clean, dry, airtight container until extraction. Care should be taken to protect the powdered plant material from moisture, excessive heat and contamination.

3.3 Extraction of Plant Material

The dried and powdered plant material of *Croton sparsiflorus* was subjected to extraction using Soxhlet extraction, using ethanol as the extraction solvent.

3.4 Extraction By Soxhlet Method

About 20 g of powdered *Croton sparsiflorus* plant material was accurately weighed and placed in the Soxhlet extraction apparatus. Approximately 100 mL of ethanol was used as the extraction solvent. The Soxhlet apparatus was assembled properly and the extraction was carried out for approximately 6 hours under controlled heating conditions. After completion of the 6-hour extraction process, the extract-containing solvent was collected and concentrated under controlled conditions to remove the solvent and obtain the ethanolic Soxhlet extract.

The concentrated extract was weighed and stored in a suitable airtight container until further phytochemical and pharmacological evaluation.



Fig: 6 Extraction Process.

3.4.1 Storage of Extracts: The extracts obtained by Soxhlet extraction were transferred into properly labelled, airtight containers and stored under suitable conditions until further analysis. The extracts were subsequently used for preliminary phytochemical screening and selected pharmacological evaluations.

3.4.2 Principle Of Percentage Yield: Percentage yield is used to determine the amount of extract obtained from a known quantity of powdered plant material after the extraction process. It represents the extraction efficiency of the selected extraction method and solvent. The percentage yield is calculated by comparing the weight of the dried extract obtained with the initial weight of the powdered plant material used for extraction.

The percentage yield is calculated using the following formula:

3.4.3 Percentage yield (% w/w)

$$\begin{aligned}
 &= \frac{\text{Weight of dried extract obtained}}{\text{Weight of powdered plant material used}} \times 100 \\
 &= \frac{4.5g}{20g} \times 100 \\
 &= 22.5\% \text{ w/w}
 \end{aligned}$$

Thus, the percentage yield of the Soxhlet extraction was found to be **22.5% w/w**

3.5 Preliminary Phytochemical Screening:

The ethanolic extracts obtained by Soxhlet extraction of *Croton sparsiflorus* were separately subjected to preliminary phytochemical screening. The purpose of the screening was to identify the major groups of phytoconstituents present in each extract. The Soxhlet extract were individually dissolved in suitable solvents and subjected to standard qualitative chemical tests for the detection of various phytochemical constituents such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids and steroids. The results obtained from both extraction methods were recorded separately and compared to determine the variation in phytochemical composition between the two extracts.

3.5.1 Ethanolic Extract Of *Croton Sparsiflorus* - EECS

Phyto-Chemical Test:



Fig: 7 Phyto-Chemical Tests.

Table: 3 Chemical Test

No.	Phytochemical Constituent	Test Performed	Positive Observation	Result
1	Alkaloids	Mayer's test	Cream or white precipitate	Present (++)
2	Alkaloids	Dragendorff's test	Orange or reddish-brown precipitate	Present (++)
3	Flavonoids	Shinoda test	Pink, red, or orange colour	Present (+++)
4	Flavonoids	Alkaline reagent test	Intense yellow colour that disappears after adding acid	Present (+++)
5	Phenols	Ferric chloride test	Bluish-black or dark green	Present

			colour	(+++)
6	Tannins	Ferric chloride test	Blue-black or green colour	Present (++)
7	Saponins	Foam test	Persistent froth after shaking	Present (+++)
8	Terpenoids	Salkowski test	Reddish-brown colour at the interface	Present (+)
9	Steroids	Liebermann–Burchard test	Green or bluish-green colour	Present (+)
10	Glycosides	Keller–Killiani test	Brown ring at the interface	Present (+)
11	Carbohydrates	Molisch's test	Violet ring at the interface	Present (++)
12	Proteins	Biuret test	Violet or purple colour	Absent (–)
13	Anthraquinones	Bornträger's test	Pink or red colour in the alkaline layer	Absent (–)

3.6 Anti-Urolithiatic Assays

Assay Of Croton Sparsiflorus

3.6.1 Nucleation Assay

This test measures the ability of the extract to prevent the initial formation of calcium oxalate crystals.

Reagents

- Calcium chloride: 5 mmol/L.
- Sodium oxalate: 7.5 mmol/L.
- Tris-HCl buffer: 0.05 mol/L.
- Sodium chloride: 0.15 mol/L.
- Adjust buffer to pH 6.5.
- Ethanolic extract of *C. sparsiflorus*.
- UV-visible spectrophotometer set at 620 nm.

Procedure

- Prepare calcium chloride and sodium oxalate solutions in Tris-HCl/NaCl buffer.
- Add 1 mL of plant extract to a cuvette or reaction tube.
- Add the required volume of calcium chloride solution.
- Start crystallization by adding sodium oxalate solution.
- Immediately mix or vortex briefly.
- Incubate at 37 °C.

- Measure absorbance at 620 nm at fixed intervals, such as every 30 seconds for 10–15 minutes.
- Run a control containing calcium chloride and sodium oxalate without extract.
- Run a standard-drug group.

Nucleation inhibition

Nucleation inhibition (%)

$$= \left[\frac{(A_c - A_s)}{A_c} \right] \times 100$$

A_c = absorbance of control;
 A_s = absorbance of sample;

3.6.2 Calcium Oxalate Crystal-Growth Assay

This test determines whether the extract prevents the enlargement of preformed calcium oxalate crystals.

Preparation Of Calcium Oxalate Seed Crystals:

- Prepare 50 mmol/L calcium chloride and 50 mmol/L sodium oxalate.
- Mix equal volumes of the two solutions.
- Heat the mixture at 60 °C for 1 hour.
- Incubate overnight at 37 °C.
- Collect the formed crystals by filtration or centrifugation.
- Wash the crystals with distilled water.
- Dry the crystals.
- Disperse them at 0.8–1.0 mg/mL in 0.05 mol/L Tris-HCl containing 0.15 mol/L NaCl at pH 6.5.

Procedure

- Add 3 mL of the calcium oxalate seed-crystal suspension to each tube.
- Add 1 mL of *C. sparsiflorus* extract at different concentrations.
- Prepare a control with 1 mL solvent instead of extract.
- Add the calcium and oxalate growth solutions according to the selected validated protocol.

- Incubate at 37 °C for 30 minutes.
- Measure absorbance at 620 nm or examine the crystals microscopically.
- Compare the increase in absorbance or crystal size with the control.

Crystal-growth inhibition

Crystal-growth inhibition (%)

$$= \left[\frac{\Delta A_c - \Delta A_s}{\Delta A_c} \right] \times 100$$

ΔA_c = change in absorbance of control;
 ΔA_s = change in absorbance of sample;

3.6.3 Calcium Oxalate Aggregation Assay

This assay evaluates whether the extract prevents already formed calcium oxalate crystals from clumping together.

Reagents

- Prepared calcium oxalate seed crystals.
- Tris-HCl/NaCl buffer, pH 6.5.
- Ethanolic extract at 100–1,000 µg/mL.
- Cystone or potassium citrate as standard.
- UV-visible spectrophotometer at 620 nm.

Procedure

- Prepare a uniform calcium oxalate crystal suspension at approximately 0.8 mg/mL in Tris-HCl/NaCl buffer.
- Add 3 mL of the suspension to each tube.
- Add 1 mL of the plant extract at the selected concentration.
- Mix rapidly and incubate at 37 °C for 30 minutes.
- Measure absorbance at 620 nm immediately after mixing and again after incubation.
- Prepare an untreated crystal suspension as the control.
- Prepare the standard-drug group under identical conditions.
- Examine a portion microscopically for crystal clumping.

Aggregation inhibition

$$\text{Aggregation inhibition (\%)} = \left[1 - \left(\frac{T_s}{T_c} \right) \right] \times 100$$

T_s = turbidity of sample;
 T_c = turbidity of control;

3.7 Anti-Inflammatory Assays

Egg Albumin Assay

The anti-inflammatory activity of unknown crude extracts can be determined in vitro for inhibition of the denaturation of egg albumin (protein).

0.2 mL of 1-2% egg albumin solution (from fresh hen's egg/ or commercially available egg albumin powder), 2 mL of sample extract or standard (Diclofenac sodium) at varying concentrations, and 2.8 mL of phosphate buffered saline (pH 7.4) were mixed to form a reaction mixture of a total volume of 5 mL.

A total volume of 5 mL of the control was created by combining 2 mL of triple-distilled water, 0.2 mL of 1-2% egg albumin solution, and 2.8 mL of phosphate-buffered saline. The reaction mixtures were then incubated at $37 \pm 2^\circ\text{C}$ for 30 min and will be heated in a water bath at $70 \pm 2^\circ\text{C}$ for 15 min. After cooling, the absorbance was measured at 280 nm by a suitable UV/Vis spectrophotometer using triple distilled water as the blank [1]. The following equation was used to determine the % inhibition of protein denaturation.

Percentage of Inhibition

$$\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

4. RESULTS AND DISCUSSION

4.1 Nucleation Assay

Table: 4 Nucleation Assay.

Concentration ($\mu\text{g/mL}$)	Nucleation inhibition (%)
100	18.42 ± 1.36
200	29.65 ± 1.51
400	43.27 ± 1.74
600	57.84 ± 1.83
800	69.36 ± 1.92
1,000	78.91 ± 2.04
Standard drug, 1,000	91.48 ± 1.27

4.2 Calcium Oxalate Crystal-Growth Assay.

Concentration ($\mu\text{g/mL}$)	Crystal-growth inhibition (%)
100	15.74 ± 1.22
200	27.83 ± 1.45
400	40.56 ± 1.68
600	54.91 ± 1.76
800	66.48 ± 1.85
1,000	75.62 ± 1.96
Standard drug, 1,000	89.76 ± 1.34

Table: 5 Calcium Oxalate Crystal-Growth Assay

4.3 Calcium Oxalate Aggregation Assay.

Concentration ($\mu\text{g/mL}$)	Aggregation inhibition (%)
100	13.86 ± 1.18
200	25.49 ± 1.39
400	38.72 ± 1.57
600	52.63 ± 1.70
800	64.27 ± 1.79
1,000	73.84 ± 1.88
Standard drug, 1,000	88.92 ± 1.29

Table: 6 Calcium Oxalate Aggregation Assay.

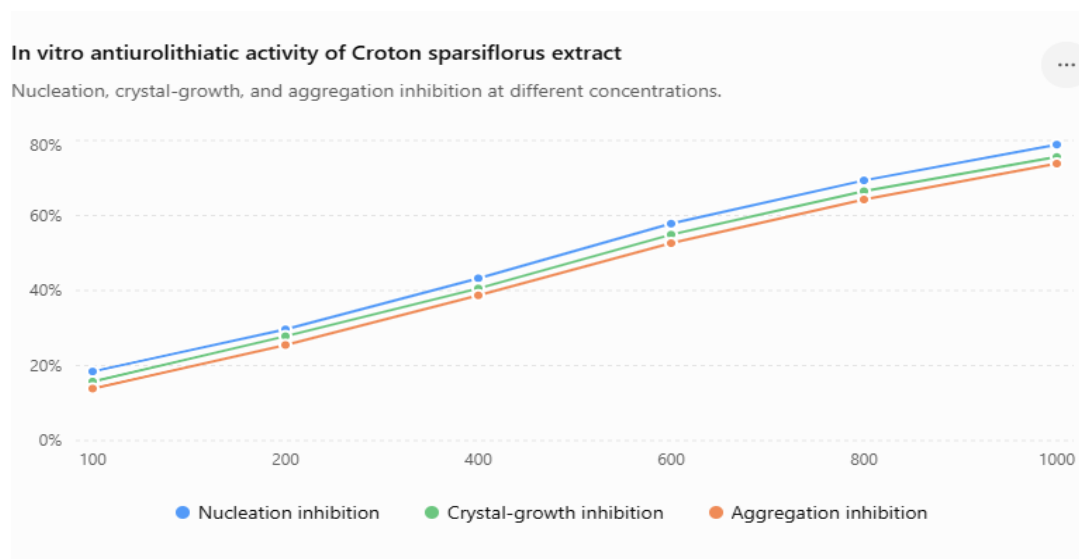


Fig: 8 Anova Graph.

ANOVA graph showing the three antiurolithiatic assays—nucleation, crystal growth, and aggregation—as mean $\pm$ SD values across extract concentrations.

Table: 7 p Value.

Assay	F statistic	p value
Nucleation inhibition	1,463.89	1.15×10^{-14}
Crystal-growth inhibition	1,407.31	1.43×10^{-14}
Aggregation inhibition	1,374.23	1.60×10^{-14}

4.4: Anti-Inflammatory Activity**Table: 8 Anti-Inflammatory Activity.**

S.NO	GROUP	OD VALUE	% OF INHIBITION
1	DISTILLED WATER	0.00	-
2	CONTROL	0.449	-
3	STANDARD	0.146	67.48
4	EECS	0.129	71.26

DISCUSSION

The present study evaluated the phytochemical profile and in vitro antiurolithiatic activity of the ethanolic extract of *Croton sparsiflorus* (EECS). Preliminary phytochemical screening revealed the presence of several biologically important secondary metabolites. Alkaloids were detected by both Mayer's and Dragendorff's tests, with a moderate positive response (++). Flavonoids showed a strong positive response (+++) in both the Shinoda and alkaline reagent tests. Phenols and saponins also showed strong positive reactions (+++), whereas tannins showed a moderate positive response (++). Terpenoids, steroids, and glycosides were detected at a lower intensity (+), while carbohydrates showed a moderate response (++). Proteins and anthraquinones were not detected in the tested extract.

The presence of flavonoids, phenolic compounds, tannins, saponins, alkaloids, terpenoids, steroids, glycosides, and carbohydrates suggests that EECS contains a diverse range of phytoconstituents that may contribute to its biological activity. In particular, phenolic compounds and flavonoids are widely associated with antioxidant and metal-chelating properties, which may influence crystal formation and growth. Other constituents, such as saponins, alkaloids, tannins, terpenoids, and glycosides, may also contribute synergistically to the observed antiurolithiatic effect. However, the phytochemical screening is qualitative and does not establish which individual constituent is responsible for the activity.

The in vitro antiurolithiatic activity of EECS was assessed using three complementary calcium oxalate assays: nucleation, crystal growth, and aggregation. In the nucleation assay, EECS demonstrated a concentration-dependent inhibitory effect. The inhibition increased from $18.42 \pm 1.36\%$ at 100 $\mu\text{g/mL}$ to $78.91 \pm 2.04\%$ at 1,000 $\mu\text{g/mL}$. This indicates that

increasing concentrations of the extract progressively reduced the initial formation of calcium oxalate crystals. Although the extract showed substantial activity at the highest concentration, its inhibition was lower than that of the standard drug, which produced $91.48 \pm 1.27\%$ inhibition at 1,000 $\mu\text{g/mL}$.

A similar concentration-dependent pattern was observed in the crystal-growth assay. EECS produced $15.74 \pm 1.22\%$ inhibition at 100 $\mu\text{g/mL}$, which increased progressively to $75.62 \pm 1.96\%$ at 1,000 $\mu\text{g/mL}$. The standard drug produced $89.76 \pm 1.34\%$ inhibition at the same concentration. These findings suggest that EECS can interfere with the enlargement of preformed calcium oxalate crystals. The inhibition of crystal growth is particularly relevant to urolithiasis because preventing the enlargement of initially formed crystals may limit the development of larger urinary stones.

The aggregation assay also demonstrated a concentration-dependent inhibitory response. Aggregation inhibition increased from $13.86 \pm 1.18\%$ at 100 $\mu\text{g/mL}$ to $73.84 \pm 1.88\%$ at 1,000 $\mu\text{g/mL}$. The standard drug showed $88.92 \pm 1.29\%$ inhibition at 1,000 $\mu\text{g/mL}$. Thus, EECS was capable of reducing the aggregation or clumping of calcium oxalate crystals, although its activity remained below that of the standard drug.

Among the three assays, nucleation exhibited the highest inhibitory activity at 1,000 $\mu\text{g/mL}$ ($78.91 \pm 2.04\%$), followed by crystal-growth inhibition ($75.62 \pm 1.96\%$) and aggregation inhibition ($73.84 \pm 1.88\%$). The consistent concentration-dependent response across all three experimental models indicates that EECS possesses activity at multiple stages of calcium oxalate stone formation. The extract was particularly effective in interfering with the initial nucleation process, while also demonstrating considerable inhibition of crystal growth and aggregation.

Statistical analysis further supported the observed differences among the tested concentrations. One-way ANOVA produced highly significant F values for nucleation inhibition ($F = 1,463.89$; $p = 1.15 \times 10^{-14}$), crystal-growth inhibition ($F = 1,407.31$; $p = 1.43 \times 10^{-14}$), and aggregation inhibition ($F = 1,374.23$; $p = 1.60 \times 10^{-14}$). Since all p values were far below 0.05, the differences among concentrations were statistically significant. These results provide strong statistical evidence for a concentration-dependent antiurolithiatic effect of EECS under the experimental conditions used.

The anti-inflammatory activity of the ethanolic extract of *Croton sparsiflorus* (EECS) was evaluated using the Egg Albumin Denaturation Assay. Protein denaturation is a well-known cause of inflammation, and substances that prevent protein denaturation are considered to possess anti-inflammatory properties. In the present study, the control group showed an

optical density (OD) value of 0.449, indicating maximum protein denaturation. The standard drug exhibited an OD value of 0.146 with 67.48% inhibition of protein denaturation. The EECS-treated group showed an OD value of 0.129 and produced 71.26% inhibition.

The results revealed that EECS exhibited a higher percentage inhibition of protein denaturation than the standard drug. The lower OD value observed for EECS indicates its strong ability to stabilize proteins and prevent denaturation. This significant inhibitory effect may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, tannins, alkaloids, and saponins, which are known to possess anti-inflammatory properties.

The findings suggest that the ethanolic extract of *Croton sparsiflorus* effectively inhibits protein denaturation and demonstrates promising anti-inflammatory activity. The observed activity may be due to the synergistic action of various phytochemicals present in the extract. Further studies involving in vivo models and isolation of active constituents are required to confirm its mechanism of action and therapeutic potential.

Overall, the findings suggest that the ethanolic extract of *Croton sparsiflorus* has promising in vitro antiurolithiatic potential & antiinflammatory. The observed activity may be associated with the combined effects of the phytochemical constituents identified in the extract. Nevertheless, the extract did not reach the inhibitory activity of the standard drug at the tested concentration. Further investigations involving dose-response modelling, isolation and characterization of active constituents, mechanistic studies, and in vivo evaluation are necessary to establish its therapeutic potential.

4. CONCLUSION

The results of the present investigation indicate that the ethanolic extract of *Croton sparsiflorus* possesses significant antiurolithiatic and anti-inflammatory activities. Preliminary phytochemical analysis revealed the presence of important secondary metabolites such as flavonoids, phenols, tannins, saponins, alkaloids, terpenoids, steroids, and glycosides, which may contribute to the observed pharmacological effects. The extract effectively inhibited calcium oxalate crystal nucleation, growth, and aggregation in a concentration-dependent manner, suggesting its potential role in preventing urinary stone formation. In addition, EECS exhibited excellent anti-inflammatory activity by significantly inhibiting protein denaturation, even showing greater inhibition than the standard drug under the experimental conditions. Although the extract demonstrated promising biological activities, its efficacy in the antiurolithiatic assays remained lower than that of the standard drug.

Therefore, further studies involving isolation and characterization of active phytoconstituents, mechanistic investigations, toxicity assessment, and in vivo evaluation are required to validate its therapeutic potential. Based on the findings of this study, *Croton sparsiflorus* may be considered a promising natural source for the development of antiurolithiatic and anti-inflammatory agents.

5. REFERENCES

1. Coe FL, Evan A, Worcester E. Kidney stone disease. J Clin Invest. 2005;115(10):2598–2608.
2. Khan SR, Pearle MS, Robertson WG, et al. Kidney stones. Nat Rev Dis Primers. 2016;2:16008.
3. Worcester EM, Coe FL. Clinical practice. Calcium kidney stones. N Engl J Med. 2010;363(10):954–963.
4. Aggarwal KP, Narula S, Kakkar M, Tandon C. Nephrolithiasis: Molecular mechanism of renal stone formation and the critical role played by modulators. Biomed Res Int. 2013;2013:292953.
5. Pareta SK, Patra KC, Harwansh RK, et al. Traditional approaches for the management of urolithiasis. J Pharmacogn Phytochem. 2011;3(1):1–12.
6. Khan A, Bashir S, Khan SR. Antiurolithic effects of medicinal plants: Results of in vivo studies in rat models of calcium oxalate nephrolithiasis. Urolithiasis. 2021;49(2):95–122.
7. Gürocak S, Küpeli B. Consumption of historical and current phytotherapeutic agents for urolithiasis. J Urol. 2006;176:450–455.
8. Butterweck V, Khan SR. Herbal medicines in the management of urolithiasis. Planta Med. 2009;75:1095–1103.
9. Bahmani M, Shirzad H, Shahinfard N, et al. Urolithiasis and medicinal plants. J Chem Pharm Res. 2016;8(2):98–105.
10. Ahmed S, Hasan MM, Mahmood ZA. Antiurolithiatic plants and their mechanisms. Pak J Pharm Sci. 2018;31(4):1423–1432.
11. Chaudhary A, Singla SK, Tandon C. In vitro evaluation of antiurolithiatic activity of herbal extracts. Urol Res. 2010;38:335–342.
12. Atmani F, Khan SR. Effects of plant extracts on calcium oxalate crystallization. Urol Res. 2000;28:404–409.
13. Soundararajan P, Mahesh R, Ramesh T. Antiurolithiatic activity of medicinal plants: A review. Int J Pharm Sci Rev Res. 2015;31:120–128.

14. Lakshmi T, Devaraj E. Antiuro lithiatic activity of phytochemical extracts: A review. *J Adv Pharm Educ Res.* 2020;10(2):1–8.
15. Bashir S, Gilani AH. Antiuro lithic effect of natural products. *Phytother Res.* 2009;23:135–141.
16. Patel PK, Patel MA. Herbal treatment of urolithiasis: An overview. *Int J Pharm Sci Drug Res.* 2012;4:1–6.
17. Verma S, Singh SP. Current and future status of herbal medicines. *Vet World.* 2008;1:347–350.
18. Harborne JB. *Phytochemical Methods*. 3rd ed. London: Chapman & Hall; 1998.
19. Trease GE, Evans WC. *Pharmacognosy*. 16th ed. London: Saunders Elsevier; 2009.
20. Kokate CK. *Practical Pharmacognosy*. 5th ed. Pune: Nirali Prakashan; 2010.
21. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. 3rd ed. Ibadan: Spectrum Books; 2008.
22. Evans WC. *Trease and Evans Pharmacognosy*. 15th ed. London: WB Saunders; 2002.
23. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol.* 2005;4(7):685–688.
24. Okwu DE. Phytochemicals and vitamin content of indigenous medicinal plants. *J Sustain Agric Environ.* 2004;6:30–37.
25. Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev.* 1999;12:564–582.